

Preparation for Sequencing of LT10 Promoter in PSB1C3

Made with Benchling

Project: Awesome Possum

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Dates: 2016-09-29 to 2016-09-30

THURSDAY, 9/29

Purpose:

To prepare 2 samples of LT10 Promoter in PSB1C3 DNA for shipment to MacroGen for sequencing. Each sample will contain DNA and 1 primer. The sequencing will be done in 2 parts separately, forward and backward.

Materials:

- Purified and Resuspended LT10 Promoter in PSB1C3 DNA
- VR Primer
- VF2 Primer
- 2x 1.5 mL eppendorf tubes
- Micropipette
- Computer
- MacroGen Bag

Protocol:

The 2 eppendorf tubes were labeled LT10 FWD and LT10 REV. A micropipette was used to deliver 5.0 μ L of DNA into each tube. Next, 5.0 μ L of VF2 primer was pipetted into the LT10 FWD tube and 2.0 μ L of VR primer was pipetted into the LT10 REV tube. Then, the tubes were deposited into a labeled MacroGen bag, and a sequencing order was generated via the vendor site. Finally, the samples were picked up and shipped to MacroGen for sequencing.

FRIDAY, 9/30

MacroGen sequencing results were received late Friday night. The sequenced DNA file was copied and compared to the expected results in Snapgene software. The sequenced DNA matched the expected results. Thus, the insertion of LT10 Pro into PSB1C3 vector using DH5a competent cells was considered successful.